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Research Article
Response of Pea Plants to Natural Bio-stimulants Under Soil Salinity Stress

[1]El-Sayed Mohamed Desoky, [2]Abdel-Rahman Mohamed Merwad and [2]Ahmed Salah Elrys

[1]Department of Agriculture Botany, Faculty of Agriculture, Zagazig University, Zagazig, Egypt
[2]Department of Soil Science, Faculty of Agriculture, Zagazig University, Zagazig, Egypt

Abstract

Background and Objective: Salinity is one of the most important abiotic stresses causing a significant reduction of crop plants yield. Most arable lands were considered unsuitable for farming due to salinity. To ameliorative the adverse effect of salinity in pea (*Pisum sativum* L.), the present study investigated the effect of integrated application of some natural bio-stimulants used as foliar spray and/or seed soaking on growth, yield and endogenous physio-chemical constituents of pea growing under soil salinity stress. **Materials and Methods:** A randomized complete block , factorial (2-factor) experiment was conducted on pea (*Pisum sativum* L.) grown on saline soil (EC = 6.11-6.17 dS m^{-1}) to assess Ascorbic Acid (AA) and *Moringa oleifera* Leaf Extract (MLE), used as foliar spray or seed soaking. Data were analyzed by a simple one way variance analysis (ANOVA). **Results:** The MLE or AA application, used as foliar spray or seed soaking, improved growth characteristics (i.e., shoot length, number of branches per plant and canopy dry weight) and physio-chemical attributes (i.e., RWC and MSI%, concentrations of chlorophylls a, b, carotenoids, total soluble sugars, free proline, contents of N, P, K and Ca) in pea plants. In addition, number of pods, pod weight and green pod yield were improved when compared with the controls (tap water foliar spray or seed soaking). Combined treatments of AA and MLE (i.e., seed soaking in AA+foliar spray with AA, seed soaking in AA+foliar spray with MLE, seed soaking in MLE+foliar spray with AA and seed soaking in MLE+foliar spray with MLE) significantly increased all above mentioned parameters compared to the control (seed soaking in tap water+foliar spray with tap water). **Conclusion:** The combined seed soaking in MLE+foliar spray with MLE treatment was found to be highly effective at improving the growth and yields of bean plants by alleviating the inhibitory effects of soil salinity stress.

Key words: Salinity, *Moringa oleifera*, ascorbic acid, growth, productivity, pea plant

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Corresponding Author: El-Sayed Mohamed Desoky, Department of Agriculture Botany, Faculty of Agriculture, Zagazig University, Zagazig, Egypt
Tel: 0020100419568

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the most popular vegetable crops grown in many of the Middle Eastern countries. Pea considers one of the main leguminous crops that are an important component of the agricultural sector in developing countries due to its ability to produce significant quantities of protein, carbohydrates and nutrient-rich seed. Its seeds contain 18-20% dry matter whose 10-12% is carbohydrate and 5-8% is protein[1].

Salinity is a serious problem in worldwide agriculture areas because it limits plant growth and productivity[2,3]. Salinity is an important abiotic stress which effect strongly on crop productivity by accumulation Na^+ and Cl^- ions and imbalance of nutrient[4]. About 800 million hectares of land, equivalent to more than 6% of total global area of Earth affected by soil salinity[5]. In the arid and semiarid regions including Egypt soil salinization caused by many factors such as, poor drainage, low rainfall, poor irrigation water which contain amount of salts that accumulate in the surface layer, high evaporation and nearness of the sea[6]. Soil salinity mitigate growth and productivity of plants due to increased in water use efficiency and in plant metabolism[7]. Plants grown under salinity conditions are basically stressed in three ways; phytotoxicity of Na^+ and Cl^- ions, decreased water potential in the rhizosphere which caused water deficit and nutrient imbalance by the reduction in the uptake and/or shoot transport[8].

Plant bio-stimulants such as *Moringa oleifera* Leaf Extract (MLE) are substances when applied to plants grown under salinity condition cane be over came the undesirable effect of stress[6]. *Moringa oleifera* is one of 13 species of genus moringa and family moringaceae which is well known vegetable in America, Africa, India, Arabia and Pakistan[9]. The MLE is a rich with antioxidant and some osmoprotectant. It's also rich natural cytokinin, zeatin, vitamin A and C, riboflavin, phenols and several minerals which making it a natural potential growth stimulant[6,10]. It has been reported that MLE accelerates growth of tomato, peanut, corn and wheat at early vegetative growth stage, improves resistance against pests and diseases, enhances plants productivity and generally increases yield by approximately 20-35%[11]. It has been observed also that MLE has antioxidant properties and considers as a source of natural antioxidants[12]. Application of MLE has beneficial effects on plant growth, photosynthetic capacity, hormonal content and antioxidative defense system activity under salt stress[13-15]. The MLE application ton plant as seed soaking or foliar spray enhanced plant tolerance to abiotic stress[6,16]. Zeatin was found to be improved the antioxidant system in plant and protect plant cells from adverse effect of ROS[17]. Application of MLE enhance antioxidant levels and activated plant defense system, increased levels of plant secondary metabolites, reduced uptake of Na^+ and/or Cl^- and improved shoot or leaf K^+. These lead to powerful seedling growth and maximizing the crop performance[14,15,18].

Ascorbic Acid (AA) is regarded as one of the most effective growth regulators which play a vital role in alleviating the adverse effect of salinity stress on growth and metabolism of many plants[19]. The AA is associated with chloroplasts the oxidative stress of photosynthesis. In addition play role in cell division and modification of protein. The AA enhance oxidative stress tolerance because it is a primary substrate in cyclic pathway of enzymatic which release H_2O_2[20]. Application of AA alleviated the adverse effect of salt stress by maintained optimum tissue water status, K^+/Na^+ ratio and enhanced antioxidant enzyme[21]. Also AA cause positive effect in protection and stabilization of photosynthetic pigments and chloroplast from oxidative damage[19,22].

The current study was designed to evaluate the potential effect of integrated application of AA and/or MLE used as foliar spray and seed soaking on growth, yield and endogenous physio-chemical constituents of *Pisum sativum* growing under moderate soil salinity stress.

MATERIALS AND METHODS

Soil analysis and preparation, plant material and experimental procedures: Two field experiments were conducted in two successive seasons (2014 and 2015) on a special farm at El-Noubaria, Egypt. In 2014 season, daily temperature ranged from 16.4-23.6°C, with average of 20.0±2.5°C. The daily relative humidity averaged 60.0±3.4% and ranged 35-81% while daily temperature in season 2015 ranged 16.9-24.2°C, with average of 20.5±2.9°C. The daily relative humidity averaged 58.0±3.7% and ranged 30-83%.

Healthy pea (*Pisum sativum* L.) cv. Master-B seeds were provided from Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. Seed were sown on 6 October in 2014 and 9 October in 2015 and were sown equivalent of 60 kg ha^{-1} to achieve the recommended planting density. Seed were selected for uniformity by choosing those of some color and equal size. The selected seeds were washed with distilled water and left in room temperature (21°C) to dry. Air dried seeds were sown, after their soaking in distilled water, *Moringa oleifera* Leaf Extract (MLE) or ascorbic acid, in hills in

rows spaced 70 cm apart. The hills were spaced 15-20 cm. apart in 3×3.5 m plots. Four seed were sown in each hole, thinning was done before the first irrigation to kept two plant per hill.

During preparation of the experimental site, farmyard manure at the rat 25 m^3, 230 kg elemental ammonium sulphate (20%) (Commercial grade), 350 kg ordinary super phosphate (15.5% P_2O_5) ha^{-1} and 120 kg potassium sulphate (48% K_2O) were added in soil. These fertilizers are commercial. Soil analysis of the experimental site in each season was carried out according to Black *et al.*[23] and Jackson[24]. The results from physical and chemical analysis of the soils are shown in Table 1. Soil EC values were 6.11 and 6.17 dS m^{-1} C in soil past extract for 2014 and 2015 seasons respectively, these EC values classed the soil being moderately saline according to Dahnke and Whitney[25]. The experiment was arranged in the randomized complete block design, with one level of each distilled water, MLE and AA with three replicates plots per treatment.

Preparation and analysis of *Moringa* Leaf Extract: Fresh leaves harvested from fully matured *Moringa oleifera* trees were air-dried, grinded and extracted. For extraction, 250 mL of ethyl alcohol 80% was added to leaf powder and the mixture was put for 4 h on a rotary shaker. Extract was purified by filtering twice through Whatman No. 1 filter paper. After purification, the extract was subjected to a rotary evaporator to fully evaporate the alcohol. Centrifugation at 8000×g for 15 min was then conducted for supernatant. Supernatant was diluted to 30 times and used to foliar spray and seed soaking applications. The chemical analysis of the extract are represented in Table 2.

Application of Ascorbic Acid (AA) and *Moringa* Leaf Extract (MLE): For seed soaking, pea seeds were soaked in distilled water, MLE using seed weight to solution volume ratio (1:5) for 1 h and AA (2 mM) at room temperature. After seed soaking, seeds were washing with distilled water and re-dried at room temperature overnight. In the morning, treated seed were sowing. Foliar spray with distilled water, MLE and AA was done at early morning with sprayer (Vol. 10 L) to rum-off three times at 15, 30 and 45 days after sowing. To ensure optimal penetration into leaf tissue, the authors added 0.1% (V/V) tween-20 to foliar sprays as surfactant. The number and timing of sprays of AA and MLE as well as concentrations and duration of soaking were based on authors preliminary study in pots.

Table 1: Physical and chemical properties of the experimental soil in two seasons

Parameters	Season 2014	Season 2015
Sand (%)	45.43	45.66
Silt (%)	30.11	29.89
Clay (%)	24.55	24.45
Soil texture	Loam	Loam
pH	7.62	7.71
EC (dS m^{-1})	6.11	6.17
Organic matter (%)	7.65	7.98
$CaCO_3$ (g kg^{-1})	60.35	61.99
Field capacity (%)	16.65	16.65
Available N (mg kg^{-1} soil)	57.98	58.42
Available P (mg kg^{-1} soil)	8.80	8.96
Available K (mg kg^{-1} soil)	97.65	98.98
Available Mg ($mmol_c$ L^{-1})	19.00	19.70
Available Ca ($mmol_c$ L^{-1})	16.60	16.70
Available Na ($mmol_c$ L^{-1})	18.10	17.80
Available Cl ($mmol_c$ L^{-1})	31.65	32.74
Available SO_4 ($mmol_c$ L^{-1})	8.19	8.35

*Soil paste, **Soil paste extract

Table 2: Chemical components of *Moringa oleifera* leaf extract (on dry weight basis)

Components	Values
Osmoprotectants	
Total amino acids (g kg^{-1} DW)	156.00
Proline (g kg^{-1} DW)	32.00
Total soluble sugars (g kg^{-1} DW)	176.00
Mineral nutrients	
Nitrogen (N) (g kg^{-1} DW)	30.80
Magnesium (Mg) (g kg^{-1} DW)	4.50
Calcium (Ca) (g kg^{-1} DW)	9.64
Potassium (K) (g kg^{-1} DW)	21.70
Phosphorus (P) (g kg^{-1} DW)	5.78
Sulphur (S) g kg^{-1} DW)	2.68
Manganese (Mn) (g kg^{-1} DW)	0.88
Iron (Fe)	1.44
Zinc (Zn)	0.39
Copper (Cu)	0.19
Antioxidants	
Salicylic acid	78.60
α-Tocopherol (µg g^{-1} DW)	34.40
Glutathione (GSH) (µmol GSH g^{-1} DW)	2.35
Ascorbic acid (Vitamin C) (mg/100 g FW)	34.80
Vitamin A (β-carotene) (mg kg^{-1} DW)	163.00
Vitamin B_1 (thiamine) (mg kg^{-1} DW)	26.00
Vitamin B_2 (riboflavin) (mg kg^{-1} DW)	210.00
Vitamin B_3 (nicotinic acid) (mg kg^{-1} DW)	800.00
Vitamin E (tocopherol acetate) (mg kg^{-1} DW)	1130.00
DPPH radical-scavenging activity (%)	81.30
Phytohormones	
Auxins (µg g^{-1} DW)	2.77
Gibberellins (µg g^{-1} DW)	2.91
Cytokinins (µg g^{-1} DW)	2.32

DW: Dry weight

Vegetative growth attributes: Fifty five days old pea plants (n = 10) from the two outer rows of each experiment unit were chose randomly, cut off at the ground level to determine shoot length (cm), number of branches per plant and canopy dry weight (g) per plant.

Estimation of green yield: At the marketable green pod stage samples were harvest from 50 random selected plants of the two outer rows in each experiment unit to estimated the number of pod per plant, average pod weight (g) and green pod yield (ton per fed).

Determination photosynthetic pigments: The photosynthetic pigments (chlorophyll a, b and carotenoids) were extracted from (0.1 g) fresh leaf by pure acetone according to Fadeel's method[26]. The pigments contents (mg g^{-1} fresh weight) were calculated using the formula of Von Wettstein[27].

Determination antioxidants activities

Enzyme extraction: The extraction was carried out according to the method reported by Vitoria *et al.*[28].

Catalase (CAT) was assayed spectro-photo-chemically according to Chance and Maehly[29].

Peroxidase (POX) was estimated by method described by Thomas *et al.*[30] .

Superoxide dismutase (SOD) activity was determined by recording the decrease in absorbance of superoxide-nitro blue tetrazolium complex by the enzyme according to Sairam *et al.*[31] .

Determination of water use efficiency: Water Use Efficiency (WUE) values as g pods per liter of applied water were calculated for different treatments after harvest according to Jensen[32] using the Eq. 1:

$$WUE = \frac{\text{Pod yield (g per pod)}}{\text{Water application (L per pod)}} \quad (1)$$

Determination of membrane stability index: The Membrane Stability Index (MSI) was determined by taking 200 mg fresh leaf material, in two sets, in test tubes containing 10 cm^3 of double distilled water. One set was heated at 40°C for 30 min in a water bath and the electrical conductivity of the solution was recorded on conductivity bridge (C_1). Second set was boiled at 100°C on a boiling water bath for 10 min and conductivity was measured on conductivity bridge (C_2). The MSI was calculated according to Rady[33] by the formula:

$$MSI\ (\%) = \left[1-\frac{C_1}{C_2}\right]\times 100 \quad (2)$$

Determination relative water content: The method described by Barrs and Weatherley[34] was used to estimate relative water content RWC.

Determination of electrolyte leakage: Total inorganic ions leaked out in the leaves was estimated by Sullivan and Ross[35] method.

Determination of proline concentration: The proline was determined according to the method of Bates *et al.*[36].

Determination of soluble sugar concentration: Total soluble sugars were extracted and determined according to Irigoyen *et al.*[37].

Determination of soluble leaf mineral: The concentrations of N, P, K^+, Na^+ and Ca were estimated as follows: digested 0.2 g of dried leaf with sulphuric acid in the presence of H_2O_2[38]. Then the mixture was diluted with distilled water. The total Na^+ and K^+ concentrations of leaf were measured directly using Flame photometer[39]. Colorimetrically, total nitrogen concentration were determined using Nessler solution according to the method described by Naguib[40]. Total phosphorus concentration was determined colorimetrically by the hydroquinone method as described by Snell and Snell[41]. Leaf Ca contents were measured using a Perkin-Elmer, Model 3300, Atomic Absorption Spectrophotometer as mentioned by Chapman and Pratt[42].

Statistical analysis: Data were analyzed by a simple one way variance analysis (ANOVA) and differences between the means were compared by Fisher's least-significant difference test (LSD) at a probability level of 95%. Significance levels were expressed as $p = 0.05$, data are significant when $p = 0.05$. Data were analyzed by Steel *et al.*[43] method using MSTAT-C[44].

RESULTS

Growth parameters (i.e., shoot length, number of branches and canopy dry weight) and green yield parameters (i.e., number of pods per plant, pod weight and pod yield ton per fed) of salt stressed Pea plants were significantly higher ($p<0.05$) than control plant by application *Moringa oleifera* Leaf Extract (MLE) and Ascorbic Acid (AA) over two successive seasons as shown in Table 3. Integrated treatment application of MLE and AA (i.e., foliar spray with MLE or AA+seed soaking with MLE and AA) significantly increased ($p<0.05$) growth parameters as well as green yield compared to control (foliar spray with distilled water+seed soaking with distilled water) the integrated treatment of foliar spray with MLE+seed soaking MLE gave the best results.

Table 3: Effect of *Moringa* Leaf Extract (MLE) Ascorbic Acid (AA) on some growth traits and green yield of pea (*Pisum sativum* L. cv. Master B) plants growing under salinity stress condition in 2014 and 2015 seasons

Foliar spray	Seed soaking	Shoot length (cm)	No. of branches	Canopy DW (g)	No. of pods (plant $^{-1}$)	Pod weight (g)	Green pod yield (ton fed^{-1})
Season 2014							
Distilled water	Distilled water	25.7^{h}	1.00^{e}	15.3^{e}	3.33^{g}	1.54^{f}	0.73^{f}
	MEL	35.3^{e}	2.33^{bcd}	19.7^{d}	5.67^{df}	2.17^{de}	1.43^{cd}
	AA	33.1^{f}	1.67^{cde}	18.4^{e}	5.00^{ef}	1.98^{e}	1.22^{de}
MLE	Distilled water	38.4^{d}	2.67^{bc}	20.8^{d}	6.67^{cd}	2.29^{d}	1.75^{bc}
	MEL	49.7^{a}	3.67^{a}	28.1^{a}	8.67^{a}	3.34^{a}	2.41^{a}
	AA	48.9^{b}	3.33^{ab}	25.8^{b}	8.33^{ab}	2.95^{b}	2.30^{a}
AA	Distilled water	29.7^{g}	1.33^{de}	17.6^{e}	4.33^{fg}	1.73^{f}	0.96^{ef}
	MEL	48.2^{b}	3.33^{ab}	25.7^{b}	8.00^{ab}	2.96^{b}	2.22^{a}
	AA	44.5^{c}	3.00^{ab}	23.0^{c}	7.33^{bc}	2.57^{c}	1.98^{b}
Season 2015							
Distilled water	Distilled water	25.9^{h}	1.33^{e}	15.9^{f}	3.67^{e}	1.79^{c}	0.82^{g}
	MEL	35.8^{e}	2.67^{cd}	20.5^{d}	6.00^{c}	2.79^{b}	1.36^{e}
	AA	33.4^{f}	2.00^{de}	19.2^{e}	5.33^{d}	2.18^{c}	1.24^{e}
MLE	Distilled water	39.3^{d}	3.00^{bc}	21.4^{d}	7.00^{b}	2.84^{b}	1.77^{d}
	MEL	50.5^{a}	4.00^{a}	29.0^{a}	9.00^{a}	3.82^{a}	2.43^{a}
	AA	49.3^{b}	3.67^{ab}	26.6^{b}	8.67^{a}	3.23^{b}	2.32^{ab}
AA	Distilled water	30.2^{g}	1.67^{e}	18.4^{e}	4.67^{d}	1.87^{c}	0.977^{f}
	MEL	49.1^{b}	3.33^{abc}	26.2^{b}	8.33^{a}	3.19^{b}	2.24^{bc}
	AA	45.2^{c}	3.33^{abc}	23.8^{c}	7.67^{b}	2.85^{b}	2.16^{c}

Values are Means±SE (n = 6) and differences between means were compared by Fisher's least-significant difference test (LSD; p = 0.05). Mean pairs followed by different letters are significantly different

Table 4: Effect of *Moringa* Leaf Extract (MLE) and Ascorbic Acid (AA) on chlorophyll content and antioxidant enzymes of pea plants growing under salinity stress condition in 2014 and 2015 seasons

Foliar spray	Seed soaking	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	CAT (A564 min^{-1} g^{-1} protein)	POX (A564 min^{-1} g^{-1} protein)	SOD (A564 min^{-1} g^{-1} protein)
Season 2014							
Distilled water	Distilled water	1.31^{e}	0.45^{g}	0.74^{h}	50.7^{i}	0.91^{f}	3.25^{f}
	MEL	1.45^{d}	0.59^{d}	0.97^{e}	63.5^{f}	1.17^{d}	6.24^{cd}
	AA	1.40^{d}	0.54^{e}	0.91^{f}	59.9^{g}	1.08^{e}	5.5^{d}
MLE	Distilled water	1.53^{c}	0.61^{d}	1.07^{d}	68.2^{e}	1.21^{d}	6.42^{bc}
	MEL	1.74^{a}	0.75^{a}	1.29^{a}	79.1^{a}	1.66^{a}	8.59^{a}
	AA	1.69^{ab}	0.71^{b}	1.18^{b}	76.5^{b}	1.48^{b}	8.05^{a}
AA	Distilled water	1.33^{e}	0.49^{e}	0.82^{g}	54.1^{h}	1.01^{e}	4.20^{e}
	MEL	1.68^{ab}	0.70^{b}	1.18^{b}	74.7^{c}	1.47^{b}	7.21^{b}
	AA	1.62^{b}	0.64^{c}	1.12^{c}	72.7^{d}	1.31^{c}	6.69^{bc}
Season 2015							
Distilled water	Distilled water	1.39^{e}	0.46^{f}	0.78^{h}	50.8^{i}	0.93^{f}	3.30^{f}
	MEL	1.51^{d}	0.62c^{d}	1.02^{e}	63.6^{f}	1.19^{d}	6.28^{c}
	AA	1.46^{d}	0.57d^{e}	0.93^{f}	60.0^{g}	1.11^{e}	5.59^{d}
MLE	Distilled water	1.59^{c}	0.65^{c}	1.11^{d}	68.2^{e}	1.23^{d}	6.46^{bc}
	MEL	1.79^{a}	0.78^{a}	1.33^{a}	79.2^{a}	1.69^{a}	8.64^{a}
	AA	1.74^{ab}	0.76^{a}	1.22^{b}	76.6^{b}	1.50^{b}	8.09^{a}
AA	Distilled water	1.37^{e}	0.52^{ef}	0.85^{g}	54.2^{h}	1.04^{e}	4.24^{e}
	MEL	1.74^{ab}	0.74^{ab}	1.21^{b}	74.8^{c}	1.49^{b}	7.26^{b}
	AA	1.68^{b}	0.67^{bc}	1.16^{c}	72.8^{d}	1.33^{c}	6.74^{bc}

Values are Means±SE (n = 6) and differences between means were compared by Fisher's least-significant difference test (LSD; p = 0.05). Mean pairs followed by different letters are significantly different, CAT: Catalase, POX: Peroxidase, SOD: Superoxide dismutase, FW: Fresh weight

All physio-chemical parameters shown in Table 4 (i.e., chlorophyll a, b, carotenoids, CAT, POX and SOD), in Table 5 (i.e., WUE, MSI, RWC, EL, proline and total soluble sugar) and in Table 6 (content of N, P, K, Na and Ca) performed the same trend of growth characteristic in both seasons 2014 and 2015.

The increased in growth characteristic and green yield were combined with increase in concentration of chlorophyll a, b, carotenoids, antioxidant enzyme, soluble sugar and free proline. Data showed that significant increased in above mention parameters under salt stress combined with foliar spray with MLE+seed soaking with AA and foliar spray with

Table 5: Effect of *Moringa* Leaf Extract (MLE) and Ascorbic Acid (AA) on water relations, proline and soluble sugar of pea plants growing under salinity stress condition in 2014 and 2015 seasons

Foliar spray	Seed soaking	WUE	MSI (%)	RWC (%)	EL (%)	Proline (mg day^{-1} FW)	Soluble sugar (mg day^{-1} FW)
Season 2014							
Distilled water	Distilled water	32.3^{g}	29.3^{i}	50.7^{h}	12.1^{a}	23.5^{f}	18.1^{f}
	MEL	52.0^{e}	40.2^{f}	64.6^{e}	9.60^{bcd}	28.0^{d}	25.8^{d}
	AA	44.4^{f}	34.9^{g}	60.2^{f}	10.3^{bc}	26.5^{e}	23.3^{e}
MLE	Distilled water	60.5^{d}	44.0^{e}	70.5^{d}	9.43^{cd}	29.0^{d}	27.8^{cd}
	MEL	90.3^{a}	64.7^{a}	90.8^{a}	8.23^{e}	33.7^{a}	34.9^{a}
	AA	83.5^{b}	60.0^{b}	84.1^{b}	8.68^{de}	32.0^{b}	33.3^{b}
AA	Distilled water	41.0^{f}	32.3^{h}	54.3^{g}	10.6^{b}	25.5^{e}	20.8^{e}
	MEL	80.3^{b}	57.0^{c}	83.2^{b}	8.65^{de}	31.7^{bc}	32.4^{b}
	AA	71.7^{c}	49.1^{d}	75.2^{c}	9.22^{cde}	30.6^{c}	29.1^{c}
Season 2015							
Distilled water	Distilled water	33.2^{g}	30.3^{i}	51.2^{h}	12.8^{a}	24.2^{g}	18.8^{g}
	MEL	52.7^{e}	41.3^{f}	64.9^{e}	10.1^{bcd}	28.6^{de}	26.3^{d}
	AA	45.1^{f}	35.9^{g}	61.0^{f}	10.8^{bc}	27.2^{ef}	23.8^{e}
MLE	Distilled water	61.0^{d}	45.0^{e}	71.1^{d}	9.76^{cde}	29.9^{d}	27.8^{d}
	MEL	90.3^{a}	65.1^{a}	91.5^{a}	8.81^{e}	34.7^{a}	35.6^{a}
	AA	84.2^{b}	60.4^{b}	84.5^{b}	9.13^{de}	33.0^{b}	33.9^{b}
AA	Distilled water	41.8^{f}	32.9^{h}	54.9^{g}	11.0^{b}	26.4^{f}	21.5^{f}
	MEL	81.1^{b}	58.1^{c}	83.9^{b}	9.1^{de}	32.6^{bc}	33.3^{b}
	AsA	72.7^{c}	49.3^{d}	76.0^{c}	9.48^{de}	31.5^{c}	29.9^{c}

Values are Means±SE (n = 6) and differences between means were compared by Fisher's least-significant difference test (LSD; p = 0.05). Mean pairs followed by different letters are significantly different, WUE: Water use efficiency, MSI: Membrane stability index, RWC: Relative water content, EL: Electrolyte leakage

Table 6: Effect of *Moringa* Leaf Extract (MLE) and Ascorbic Acid (AA) on N, P, K, Na and Ca of pea (*Pisum sativum* L. cv. Master B) plants growing under salinity stress condition in 2014 and 2015 seasons

Foliar spray	Seed soaking	N (mg g^{-1} FW)	P (mg g^{-1} FW)	K (mg g^{-1} FW)	Na (mg g^{-1} FW)	Ca (mg g^{-1} FW)
Season 2014						
Distilled water	Distilled water	20.9^{h}	2.70^{g}	18.0^{f}	9.90^{a}	2.16^{h}
	MEL	27.5^{e}	3.25^{de}	21.1^{d}	7.85^{d}	2.64^{e}
	AA	25.6^{f}	3.14^{ef}	19.8^{e}	8.43^{c}	2.46^{f}
MLE	Distilled water	29.0^{d}	3.48^{d}	22.1^{cd}	7.49^{e}	2.83^{d}
	MEL	33.3^{a}	4.58^{a}	26.2^{a}	6.22^{h}	3.23^{a}
	AA	31.9^{ab}	4.2^{6b}	25.2^{ab}	6.66^{g}	3.08^{b}
AA	Distilled water	23.7^{g}	2.95^{fg}	19.6^{e}	9.54^{b}	2.27^{g}
	MEL	30.8^{bc}	4.19^{b}	24.8^{b}	6.92^{f}	3.05^{bc}
	AA	29.9^{cd}	3.80^{c}	23.1^{c}	7.18^{f}	2.97^{c}
Season 2015						
Distilled water	Distilled water	21.6^{g}	2.89^{c}	18.8^{f}	9.93^{a}	2.18^{h}
	MEL	28.1^{d}	3.87^{b}	22.2^{d}	7.89^{d}	2.66^{e}
	AA	26.3^{e}	3.70^{b}	20.3^{e}	8.46^{c}	2.49^{f}
MLE	Distilled water	29.7^{c}	3.77^{b}	22.7^{cd}	7.52^{de}	2.85^{d}
	MEL	34.3^{a}	4.94^{a}	27.1^{a}	6.26^{h}	3.25^{a}
	AA	32.5^{b}	4.75^{a}	25.9^{ab}	6.69^{g}	3.10^{b}
AA	Distilled water	24.2^{f}	3.13^{c}	20.4^{e}	9.57^{b}	2.29^{g}
	MEL	31.7^{b}	4.71^{a}	25.1^{b}	6.96^{fg}	3.07^{bc}
	AA	30.4^{c}	3.95^{b}	23.7^{c}	7.22^{ef}	2.99^{c}

Values are Means±SE (n = 6) and differences between means were compared by Fisher's least-significant difference test (LSD; p = 0.05). Mean pairs followed by different letters are significantly different, FW: Fresh weight

AA+seed soaking with MLE while combined treatment application of MLE foliar spray+MLE seed soaking gave the best results during two successive seasons 2014 and 2015.

In addition combined treatments was increased WUE, MSI, RWC, N, P, K and Ca under salt stress condition as compared to control. In this respect the best combined treatment foliar spray with MLE+seed soaking with MLE was decreased EL and Na of Pea plants under salt stress during two seasons as compared to control.

DISCUSSION

In the present study the adverse effect of soil salinity decreased growth and productivity of pea plants (Table 3). The reduction of growth and productivity could be attributed to the osmotic effect of salinity stress which caused decreased of growth promoters, increase of growth inhibitors and disturbance of water in plants grown under salt stress. These inhibitory effects of salinity lead to stomatal closure, reduced

photosynthesis, disturbance in ionic homeostasis, accumulation of toxic Na^+, Cl^- and finally inhibit growth and productivity[33,45,46].

Pea seed and plant treated with Ascorbic Acid (AA) and *Moringa oleifera* Leaf Extract (MLE) used as foliar spray and seed soaking significantly enhanced plant growth and productivity under the adverse effect of soil salinity stress. Salinity stress causes oxidative damages in plant cells. One of compounds which has antioxidative characteristic are Ascorbic Acid (AA). This compound can decrease the harmful drought effects in plants under stress[47]. The AA treatments ameliorated the negative effect of salt stress on growth and productivity this could be attributed to the biochemical function of AA have been divided into four categories (1) enzyme cofactor for hydroxylase enzymes involved in the synthesis of hydroxyproline-rich glycoproteins, cell wall structural proteins, (2) Antioxidant, change the lipophilic antioxidant α-tocopherol, vitamin E, (3) Electron transport, acts as an *in vitro* electron donor and acceptor in transmembrane electron transport and (4) Oxalate and tartrate synthesis[48-51].

Analysis of MLE indicated that this extract can be used as a plant biostimulant. The MLE contain essential macro and micro elements, antioxidant such as proline, ascorbic acid, total soluble sugar and phytohormone such as indol-3-acetic acid (IAA), gibberellins (GA) and zeatin as a cytokinin (Table 2).

Many researches indicated that MLE used to adverse the effect of salinity stress on growth and productivity in different crops[6,14-16]. From results of this study application of MLE enhanced growth parameters this might be due to the enhanced mobilization of germination related metabolites/inorganic solutes such as zeatin, IAA, Ca and K presented in MLE to growing plumule or enhanced in activity of amylase enzyme and reducing sugar, attributed to absorption of water and increase cell elongation which increased plant growth[52]. Data in the present study (Table 4) showed that pea plants grown under soil salinity condition significantly decreased chlorophyll a, b and carotenoids. The change in leaf chlorophyll content might be due to degradation of chlorophyll or reduction in biothensis under salinity stress condition. It is also showed that salinity stress break down of chloroplast including plastid envelop thylakoids[53], effect of toxic Na^+ on photosynthesis apparatus or salt induced oxidative damage[54]. Under saline soil condition chlorophyll a, b and carotenoids were increased by application of AA and MLE seed soaking and/or foliar spray. It is suggested that foliar application of ascorbic acid protected photosynthetic pigments from oxidative damage by salt stress. The role of ascorbate in photosynthesis namely as a scavenger of the toxic species of activated oxygen and a cofactor of photosynthetic electron transport coupled to photophosphorylation[55]. This is may be attributed to cytokinins containing-MLE, according to the fact that moringa is a rich source of zeatin[56]. Cytokinins are generally considered to be antagonists of ABA, with the two hormones having opposing effects in several developmental processes. Zeatin-type cytokinin acts as a direct free radical scavenger or it may involve in antioxidative mechanism related to the protection of purine breakdown[57].

In the present investigation activities of antioxidant enzymes CAT, POX and SOD in pea plants were increased under salt stress as well as after treated with AA and/or MLE as seed soaking or foliar spray (Table 4) these increased in levels of antioxidant enzymes might be attributed to their role to help plants resistance against oxidative damage. Application of AA increased antioxidant enzymes of wheat plants under salt stress condition[58].

Seed soaking and foliar spray with MLE enhanced activates of antioxidant enzymes this may be due to that MLE is rich in some antioxidant (i.e., proline and ascorbic acid) and phytohormones[14]. The increased in antioxidant enzymes enhanced resistance to oxidative stress[59].

Soluble sugar and proline are significantly increased in salt stressed pea plants by application AA and/or MLE as seed soaking or foliar spray (Table 5). The increased in soluble sugar and proline supported the antioxidant system in plants to enable them to tolerate salt stress[60]. To avoid oxidative damage caused by stress, plant have developed antioxidant system, among accumulation proline which induced resistance mechanism against salinity stress[61]. The accumulation of proline has been considered as a carbon and nitrogen source for rapid recovery from stress and growth, stabilizer for membranes and some macro molecules and also a free radical scavenge[62]. Application of AA significantly increased soluble sugar and proline these results agreed with the finding by Hassanein *et al.*[63]. Soil salinity decreased in water relations such as RWC, WUE and MSI but increased leaf Electrolyte Leakage (EL) (Table 5). while application of AA and/or MLE significant increased RWC, WUE and MSI and decreased EL, this trend was more effective in salt stress tolerances when used AA and MLE as seed soaking combined with foliar spray, the highest increased in water relations when using MLE seed soaking combined with foliar spray. Electrolyte leakage enable cell membrane damage to be assessed when plant are subjected to salinity stress. Preserving integrated of cellular membranes under salt stress in considered an integral part of salinity tolerance mechanism[64]. The RWC is an important measure of physiological plants water status[65]. In

this respect application of MLE enhanced water relations such as RWC and WUE further more MLE positively modify the membrane stability index under salinity stress[14].

The increased accumulation of Na^+ ions in salt-stressed plants can disturb or upset the ionic balance, inducing a nutritional imbalance due to the blockage of other cations such as N, P, K and Ca tested in the present study or anions such as NO_3^- and thereby the induction of nutritional deficiency symptoms[31]. The maintenance of the ionic homeostasis under salt stress is prerequisite to protect the plant against the build-up of toxic ions, with K^+ and Ca^{2+} accumulating and Na^+ reaching the minimum content in bean leaves (Table 6).

Salt stress tolerance in pea plants, in this study, was stimulated with the elevated antioxidant system, including non-enzymatic antioxidants (i.e., carotenoids, free proline and soluble sugar) by the application of MLE singly or in combination with AA. *Moringa oleifera* leaves is a rich source in zeatin, minerals and other Phytohormones, so the effectiveness of MLE in mitigating salinity stress. Finally this study recommended that application of MLE and AA as seed soaking and/or foliar spray could alleviate the adverse effect of salinity on pea plants.

CONCLUSION

The present study indicated that, even if oxidative stress stimulated pea plants grown under salinity stress. Soil salinity decreased all growth parameters, productivity, photosynthetic pigments, neutral uptake and antioxidant system which involved as one of responsible factors for salinity tolerance in plants. Seed soaking and/or foliar spray with *Moringa* Leaf Extract (MLE) and Ascorbic Acid (AA) could protect the plants against disadvantages by salt stress. Foliar spray with MLE+seed soaking with MLE was the most effective treatments in providing Pea plants with salt tolerance when grown under salinity stress. Therefore used combination (MLE foliar spray+MLE seed soaking) to stimulate plant growth and productivity under soil salinity stress and may have significant effective application.

SIGNIFICANT STATEMENTS

This study discovers the possible synergistic effect of *Moringa oleifera* Leaf Extract (MLE) and Ascorbic Acid (AA) used as seed soaking or foliar spray in support the antioxidant system of pea (*Pisum sativum* L.) plants against the adverse effect of soil salinity stress. Combinations of AA and MLE as seed soaking or foliar spray may be more effective to improvement antioxidant system of pea plants under salinity stress condition. This study will help the researcher to ameliorative salinity stress on plants by application MLE and/or AA and help them to understand the role of MLE and/or AA on scavenging free radicals and ion exchange.

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